Immune function in hyperbaric environments, diving, and decompression

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Brenner I, Shephard RJ, Shek PN. Immune function in hyperbaric environments, diving, and decompression. Undersea Hyper Med 1999; 26(1):27-39.—The purpose of this review is to examine the influence of exposure to hyperbaric oxygen (HBO₂), deep diving, and decompression on various facets of the immune response. Potential changes during exposure include a decrease in the CD4⁺:CD8⁺ ratio, a decreased proliferation of lymphocytes, and an activation of neutrophils with migration to regions of high oxygen pressure. There may also be an activation of the complement cascade during decompression. Clinical indicators of overall immune suppression include a decreased response to antigens, a weakening of autoimmune responses, and a slower rejection of allografts. In professional divers, immune changes are at least partially offset by acclimatization, and seem to have little clinical significance. However, patients receiving HBO₂ are a more vulnerable group, in their case, exposure may impair immune surveillance, and a careful monitoring of immune function may be important to the success of treatment.

CD4⁺:CD8⁺ ratio, complement, infection, neutrophils, T cells

The purpose of this review is to examine the respective impacts of hyperbaric oxygen (HBO₂) and saturation diving on immune function. There has been much discussion whether exposure to such environments could lead to either a clinically significant modification of overall immune responses (1-4) or local inflammatory responses at sites of bubble formation (5).

Potential mechanisms of general immunosuppression might include the physiologic and toxic effects of high partial pressures of oxygen and the resulting increased generation of reactive species, a response to the tissue trauma incurred during decompression and subsequent repair processes, a redistribution of blood flow induced by mechanical pressures, catecholamine secretion or intravascular bubble formation, and more general effects of temperature change, altered perceptual cues, anxiety, "stress", and related hormonal responses. Unfortunately, most authors have failed to distinguish clearly among this multiplicity of stimuli, and although many reports describe changes in individual elements of the immune system, it remains difficult to draw strong conclusions regarding the underlying physiopathologic mechanisms. Nevertheless, it seems important to establish the nature, extent, and clinical significance of any immune changes that may be induced by hyperbaric treatment and diving.

Environmental conditions differ substantially between the two situations. A typical hyperbaric treatment lasts for no more than 2 h. The total ambient pressure usually does not exceed 280 kPa, but nevertheless the PO₂ is high. In contrast, a saturation dive may last for as long as 30 days. The PO₂ is usually lower (40-50 kPa), but exposure to a very high total ambient pressure (5 or more MPa) necessitates a sophisticated decompression schedule. Moreover, use of helium-oxygen mixtures may present a challenge to mechanisms of heat balance. Thus we may anticipate not only similarities but also differences in immune responses between the two situations.

This review first summarizes reported changes in cellular and humoral components of the immune system, and then considers their clinical significance in terms of overall immune responsiveness. Possible mechanisms underlying the observed changes are then discussed briefly. A final section points to avenues for future research.

DIRECT EVIDENCE OF IMMUNOSUPPRESSION

Direct evidence of immunosuppression may be sought from changes in the total numbers of circulating leukocytes, in the rate of lymphocyte proliferation, in the numbers and functional capacity of individual leukocyte subsets, and in the production or plasma concentrations of soluble components (cytokines, immunoglobulins, complement and acute phase reactants).

Technical considerations: In animals, leukocytes can be collected from sites such as the spleen (6), but in humans, cell counts are usually determined in specimens of peripheral blood; changes in circulating counts may thus reflect...
a redistribution of cells rather than a true change in numbers or function (7). Further, cell numbers and the concentrations of soluble components can be altered acutely by changes in blood or plasma volume. Particularly in animals, diving can induce substantial changes in hematocrit and red cell count (8,9).

When examining leukocyte function, it is also important to ensure that cells do not become damaged during their removal from the hyperbaric chamber. This can be assured by using a small service lock, with decompression of blood specimens at a rate of less than 100 kPa · min⁻¹ (10).

Cellular changes: Feldeier et al. (1) found no changes in the immune responses of healthy volunteers who were exposed to 240 kPa of O₂ for 90 min on 20 successive days, but others have noted cellular changes in both animals and humans in response to single or repeated hyperbaric sessions (2,3,6,11–13). Some authors have also reported adverse effects from actual or simulated deep-sea diving (10,14). However, immunosuppression has not always been observed in human subjects (15).

Dominant changes include a decrease in CD4⁺ count and CD4⁻:CD8⁺ ratio, a suppression of macrophage function, a neutrophilia and an eosinopenia. The changes in circulating cell count are quite transient [for example, <24 h after a single 90-min exposure of humans or rats to 280 kPa of O₂ (12)], suggesting that a redistribution of leukocytes between the circulation and peripheral depots is largely responsible.

Total leukocyte count: Several investigators have seen no change in total leukocyte count after air (16–18) or He–O₂ saturation dives in small-scale, uncontrolled human trials (10,19,20) (Table 1). Even 12 days of repeated air diving to a depth of 45.7 meters of sea water (msw) (150 fsw) did not change total leukocyte count (21). However, one report described a significant decrease of leukocyte count in a small group of four divers following the first of five simulated dives to 39.6 msw (130 fsw); this particular dive was for 10 min, with no subsequent decompression phase (22). Sano and associates (22) suggested that the extent of hemolytic changes in subsequent dives was small because the safety of decompression procedures was enhanced beyond the standard US Navy diving schedule.

One report found a correlation between white cell counts, either before or after a simulated dive, and the bubble score during decompression (16). It was suggested that in some unexplained manner a high white cell count had predisposed to decompression illness. Several studies have shown a sequestration of white cells at the bubble–blood interface (5,23, 24), but this seems a secondary phenomenon, possibly triggered by a local denaturation of proteins during the decompression phase (5,25).

An increased leukocyte count has sometimes been observed after a dive. This has been attributed to the development of an infection, possibly precipitated by the dive (26,27), although a secondary reaction to tissue trauma could also be involved.

Lymphocyte proliferation: Lymphocyte proliferation is a fundamental component of adaptive responses to infection, influencing both the secretion of cytokines and the production of immunoglobulins.

In animals, adverse effects of HBO₂ seem fairly well established. Exposure of mice to 100% O₂ reduces lipopolysaccharide-induced spleen cell proliferation (28). Likewise, repeated exposure to HBO₂ reduces the proliferative response of murine splenic lymphocytes to mitogens (15). In humans, phytohemagglutinin (PHA)-induced lymphocyte proliferation is depressed by air dives to 39.6 msw (130 fsw) (22) (Table 2), although it remains to be established which aspect of the diver’s environment is responsible for this change.

In contrast, a study of space-shuttle candidates noted that individuals who were vulnerable to gas emboli during decompression showed an enhanced response to the mitogen PHA before decompression when they were compared with those who did not develop venous embolism (29).

T cells: The T cells carry the major responsibility for cell-mediated immune defense. The circulating CD4⁺ count and the CD4⁺:CD8⁺ ratio are thus critical indices of immune health (Table 3). An increased susceptibility to infections and certain types of neoplasms is likely if the peripheral blood CD4⁺ count falls below 200 × 10⁶ cells liter⁻¹, or the CD4⁺:CD8⁺ ratio is less than unity.

A single 90-min exposure to HBO₂ (280 kPa) induced a decrease of the circulating CD4⁺:CD8⁺ ratio in both humans (11) and rats (12). This was apparent immediately post-exposure, and was only partially reversed 24 h later. It was due mainly to an increase in the absolute numbers of circulating CD8⁺ (suppressor) cells (11,30). Cell migration seems responsible, since animal studies show an increased CD4⁺:CD8⁺ ratio in the lungs and the lymph nodes, but a decreased ratio in the spleen (12). One brief report on a small number of subjects suggested that a succession of five short, simulated dives to 39.6 msw (130 fsw) induced no change in CD4⁺:CD8⁺ ratio on Day 1, but a significant increase in CD4⁺:CD8⁺ ratio as the dives were repeated (22). In better agreement with the hyperbaric data, simulation of a 30-day, 440-m saturation dive induced a decline in the percentage of CD3⁺ cells from 68.0 to 55.8% by Day 8, a fall of CD4⁺ count from Days 4 to 15, and a CD4⁺:CD8⁺ ratio below the critical value of 1.0 during and after decompression (10,31). Others have described the development of similar decreases in the CD4⁺:CD8⁺ ratio over the course of a training program that included repeated air dives (30).
### Table 1: Changes in Leukocyte Count With Deep Diving and Decompression

<table>
<thead>
<tr>
<th>Reference</th>
<th>Blood Sample Times</th>
<th>Subject No. (Age)</th>
<th>Depth</th>
<th>Duration</th>
<th>Decompression</th>
<th>Leukocytes</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Eosinophils/Basophils</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bühlmann et al. (19)</td>
<td>pre post</td>
<td>3</td>
<td>31 atm abs</td>
<td>81 h</td>
<td>88 h—principle of multiple gas decompression</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(oxygen-helium compression chamber)</td>
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<tr>
<td>Eckenhoff &amp; Hughes (21)</td>
<td>pre 2 h post</td>
<td>15 (29.2 ± 6.6)</td>
<td>150 ft</td>
<td>30 min</td>
<td>8 min @ 20 fswg 24 min @ 10 fswg</td>
<td></td>
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<tr>
<td>(repetitive diving, over 12 days)</td>
<td>(air diving)</td>
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<td>each (over 12 days)</td>
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<tr>
<td>Goad et al. (16)</td>
<td>1 wk before pre</td>
<td>18 (25–44)</td>
<td>210 fsw</td>
<td>50 min</td>
<td>170/30 table</td>
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<td></td>
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<tr>
<td>(air diving)</td>
<td>30 min post</td>
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<tr>
<td>(air diving)</td>
<td>45 min post</td>
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<tr>
<td>(air diving)</td>
<td>24 h post</td>
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<tr>
<td>(air diving)</td>
<td>48 h post</td>
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<tr>
<td>Jacey et al. (48)</td>
<td>pre 1 h post</td>
<td>19 (34.4 ± 7.0)</td>
<td>188 fsw</td>
<td>40–45 min</td>
<td>US Navy standards</td>
<td></td>
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<td></td>
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<tr>
<td>(air diving, single and double dives)</td>
<td>1 day post</td>
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<td>3 days post</td>
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<td>7 days post</td>
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<tr>
<td>Philp et al. (5)</td>
<td>pre post</td>
<td>15 m (1 F) (24–41)</td>
<td>300 ft</td>
<td>10 min</td>
<td>standard</td>
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<tr>
<td>(air diving)</td>
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<td></td>
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</tr>
<tr>
<td>Seno et al. (22)</td>
<td>pre post (Day 3)</td>
<td>6</td>
<td>dive 1: 10</td>
<td>10 min</td>
<td>none</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(repetitive diving, over 3 days)</td>
<td>post (Day 5)</td>
<td></td>
<td>130 fswg</td>
<td></td>
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<tr>
<td>(following dives 1–3)</td>
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<td>dive 2: 25</td>
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<td>130 fswg</td>
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<td>dive 3: 25</td>
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<td></td>
<td></td>
<td>130 fswg</td>
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</tr>
<tr>
<td>Seno et al. (22)</td>
<td>pre (C) bottom (B)</td>
<td>4 (32.0 ± 5.6)</td>
<td>130 ft</td>
<td>10 min</td>
<td>none</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(air diving)</td>
<td>surfing (S)</td>
<td>5 (34.0 ± 6.8)</td>
<td>130 ft</td>
<td>25 min</td>
<td>10 min @ 10 ft</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>2 h post (2 h)</td>
<td>5 (37.4 ± 5.9)</td>
<td>130 ft</td>
<td>25 min</td>
<td>5 min @ 20 ft &amp; 10 min @ 10 ft</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>6 h post (6 h)</td>
<td>5 (36.8 ± 6.3)</td>
<td>130 ft</td>
<td>25 min</td>
<td>10 min @ 10 ft</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>130 ft</td>
<td>28 min</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Dive and Decompression:
- US Navy standards
- Single dive: 1 h post
- Double dive:
  - 1 h post
  - Eosinophils:
  - 1 h post

Leukocytes and Differentials:
- Band neutrophils:
  - Basophils:

- Notes:
  - @ S: 2–6 h
  - @ S: 6 h

Table 2: Changes in Lymphocyte Proliferation Rate With Deep Diving and Decompression

<table>
<thead>
<tr>
<th>Reference</th>
<th>Blood Sample Times</th>
<th>Subject No. (Age)</th>
<th>Dive</th>
<th>Depth</th>
<th>Duration</th>
<th>Decompression</th>
<th>Immune Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krzyak &amp; Tchorzewski (30) (air diving)</td>
<td>pre training post training</td>
<td></td>
<td></td>
<td>2 atm abs 6 atm abs</td>
<td></td>
<td></td>
<td>delayed skin hypersensitivity test</td>
</tr>
<tr>
<td>Seno et al. (22) (repetitive diving, over 3 days)</td>
<td>pre post, Day 3 post, Day 5 (after Days 1–3)</td>
<td>6</td>
<td>dive 1: 130 fswg</td>
<td></td>
<td>10 none</td>
<td>none</td>
<td>lymphocyte proliferation</td>
</tr>
<tr>
<td>Seno et al. (22) (bottom B, surfacing S)</td>
<td>pre (C) 2 h post (2 h) 6 h post (6 h)</td>
<td>4 (32.0±5.6) 5 (34.0±6.8) 5 (37.4±5.9)</td>
<td>130 ft 130 ft 130 ft</td>
<td>10 min @ 10 ft 10 min @ 10 ft 10 min @ 10 ft</td>
<td></td>
<td></td>
<td>lymphocyte proliferation</td>
</tr>
</tbody>
</table>

Hyperbaric conditions also modify certain aspects of T cell function. The surface expression of receptors for the cytokine interleukin-2 (IL-2) is increased on T cells at all sampling sites, although cells in the lungs show a decreased expression of αβ major histo-compatibility (MHC) receptors. In support of the view that lymphocytes are activated by diving, one study noted that the proportion of “atypical” lymphocytes increased over 12 days of successive dives to 45.7 m (150 feet) (21). Likewise, exposure of rats to HBO₂ increased the proportion of circulating T cells that carried newly synthesized HLA-DR, a marker of cell activation (11). T cells from mice treated with HBO₂ also fail to react normally with B cells from untreated mice (6).

A single HBO₂ treatment (240 kPa × 90 min) had no effect on NK cell count (11). Likewise, no significant changes in circulating NK cell count were seen over a 30-day saturation dive to 440 m (10). A significant increase of NK cell count developed during and after a diving training program (30), although it was not clearly demonstrated that the diving itself was responsible for this change. The increase of NK cell count would help to compensate trained divers for any immediate suppression of cytokine secretion and thus of NK cell activity during a dive.

**Monocytes and macrophages:** Macrophages are important both to phagocytosis and (through their secretion of IL-1) to initiation of the cytokine cascade. Their effectiveness can be influenced by changes in either the cell count per unit volume of blood or the activity of individual cells. Any changes in the circulating monocyte count during diving or exposure to HBO₂ are small and inconsistent. Exposure to HBO₂ may induce a transient increase in monocyte count (11), with an increased proportion of HLA-DR-bearing cells (11). Eckenhoff and Hughes (21) noted that the percentage of monocytes peaked at Day 6 of a 12-day series of dives to 45.7 msw (150 fsw), returning to resting levels as the series continued (21). A series of dives to 39.6 msw (130 fsw) also found an increase in monocyte numbers on Day 2, but a decrease by Day 4, when a more extended pattern of decompression was adopted (22).

Hyperbaric environments adversely affect the function of macrophages in both the lungs (32) and the peritoneal cavity (32). There is a marked decrease in the production of both IL-1 (which would otherwise tend to trigger the immune cascade) and prostaglandin (which suppresses NK function) (3). Circulating macrophages also show a depression of function with exposure to HBO₂ (3,33–35). On the other hand, the phagocytic activity of murine splenic macrophages shows little change (3).

Possibly, the intracellular production of free radicals exceeds the protective capacity of macrophage oxidases, although in experienced divers repeated exposure to high concentrations of free radicals would be likely to induce a compensating increase in the expression of oxidases (36,37). Alternatively, macrophage function may be suppressed by stress-related corticosteroid secretion (4); in this
### Table 3: Changes in Lymphocyte Subsets With Deep Diving and Decompression

<table>
<thead>
<tr>
<th>Reference</th>
<th>Blood Sample Times</th>
<th>Subject No. (Age)</th>
<th>Dive</th>
<th>Lymphocyte Subsets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Depth</td>
<td>Duration</td>
</tr>
<tr>
<td>Krzyak &amp; Tchorzewski (30) (air diving)</td>
<td>pre</td>
<td></td>
<td>2 atm abs</td>
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<tr>
<td></td>
<td>post</td>
<td></td>
<td>6 atm abs</td>
<td></td>
</tr>
<tr>
<td>Seno et al. (22) (repetitive diving, over 3 days)</td>
<td>pre</td>
<td>6</td>
<td>dive 1:</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>post (Day 3)</td>
<td></td>
<td>130 fswg</td>
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<tr>
<td></td>
<td>post (Day 5)</td>
<td></td>
<td>25</td>
<td></td>
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<tr>
<td></td>
<td>(following dives 1-3)</td>
<td></td>
<td>130 fswg</td>
<td></td>
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<td>25</td>
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<td></td>
<td></td>
<td></td>
<td>130 fswg</td>
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<tr>
<td>Shinomiya et al. (10) (air diving)</td>
<td>pre</td>
<td>5 (30 ± 8.1)</td>
<td>440 m</td>
<td>6 d 3 h</td>
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<tr>
<td></td>
<td>Day 4</td>
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<td></td>
<td>Day 6</td>
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<td></td>
<td>Day 15</td>
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<tr>
<td></td>
<td>Day 22</td>
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</table>
case, habituation would probably diminish the effect as dives were repeated. A further potential factor, particularly during the use of helium gas mixtures, is a hypothermia-induced secretion of catecholamines (14).

**Neutrophils:** The number and phagocytic activity of neutrophils are important in countering bacterial infection, and in removing damaged tissues which have been identified and prepared as targets by the group of substances known as opsonins (antibodies, complement, and acute phase proteins). Both diving and hyperbaric conditions are associated with increased neutrophil counts. The cells also become activated and migrate to tissues where high PO2 or bubble formation, or both are inducing an inflammatory reaction or protein denaturation, or both.

Eckenhoff and Hughes (212) noted an increase in the number of circulating immature ("Band") neutrophils immediately before a series of 45.7 m (150 ft) dives. The neutrocycosis resolved as the experiment progressed, suggesting that "stress" had led to an anticipatory catecholamine-induced mobilization of neutrophils. Shinomiya and associates (10) also observed an increased neutrophil count during a 30-day saturation dive to 440 m, with a gradual decrease in neutrophil numbers during subsequent decompression. In another study (22), repeated diving provoked a significant neutrophilic leukocytosis, although here the response may have been secondary to diving-induced micro-injuries.

Neutrophils can be activated by pulmonary injuries associated with exposure to O2 pressures as low as 100 kPa, both in vivo and in vitro (38), probably through the action of the complement fraction C5a, leukotriene B4, platelet activating factor, or macrophage-generated IL-8. These substances also play an important chemotactic role, and during prolonged hyperoxia they attract many neutrophils to areas of the body where the PO2 and resultant tissue damage are greatest (particularly the lungs) (39-43). Some authors have suggested that the intrapulmonary accumulation of neutrophils is mediated in part by an up-regulated expression of intercellular adhesion molecule-1 (ICAM-1) on the inflamed alveolar epithelium. In mice, lung ICAM-1 mRNAs are increased by hyperoxia (44), and the accumulation of neutrophils after exposure to >95% O2 for 60-84 h can be reduced experimentally by the administration of an anti-ICAM-1 antibody (45). On the other hand, exposure to HBO2 (2.8-3.0 atm abs) down-regulates the expression of beta-2 integrins on the neutrophils, limiting the firm endothelial adherence which precedes extravascular migration of these cells (46,47).

**Eosinophils:** The eosinophils act against parasites that are too large to be ingested by other types of leukocyte. One report suggests that diving decreases circulating cell numbers. Jacey and associates (48) exposed subjects to a simulated 57.3 msw (188 fsw). When two such dives were performed with a 3-day interval, decompression from the second dive was followed by a pronounced eosinopenia.

**Basophils:** The basophils contain granules that are rich in histamine. They are particularly active in inflammatory reactions. Eckenhoff and Hughes (21) observed a progressive increase in the percentage of basophils over 12 days of 45.7 m (150-ft) dives. They suggested this might reflect a reaction to denatured protein at sites of bubble formation.

**Soluble components:** Soluble components of the immune response include cytokines, immunoglobulins, complement, and acute-phase reactants.

**Cytokines.** Hyperbaric oxygen (250 kPa, 1 h·day-1 for 5 days) significantly decreased the IL-1 production of murine splenic macrophages (3). Perhaps in part as a consequence of this change, decreases in serum IL-2 concentrations and in vitro production of IL-2 by isolated peripheral blood mononuclear cells have also been reported (3,13), with reduced serum levels of soluble IL-2 receptor (13). However, concentrations of IL-6 have shown no significant change (3). Taken together, these changes generally point to immunosuppression.

In vitro studies have further shown that O2 pressures as low as 100 kPa can more than double monocyte production of the chemotactic cytokine IL-8 (38). Treatment with lipopolysaccharide (LPS) further boosts the production of IL-8 by a factor of 2-3 (38).

**Immunoglobulins:** Decreases in serum and mucosal immunoglobulin concentrations predispose to infection. The in vitro production of immunoglobulins is decreased after several days of HBO2 treatment (3,6), and serum IgG levels fall (49). One factor contributing to this response may be an associated decrease in macrophage IL-1 secretion, and thus an impaired proliferation and maturation of B cells (6). However, serum IgG has a fairly long half-life (about 3 wk), so that other factors such as a redistribution of proteins must also contribute to the changes in serum concentrations.

Complement is essential for the initiation of inflammation and is also involved in opsonization, the preliminary coating of proteins that precedes their ingestion by phagocytes. Complement normally circulates in inert form, but a cascade of activation can be initiated by an antibody or an acute-phase protein. C3 is one of some 30 complement proteins. Cleavage by either the classical or the alternative pathway generates C3a and C3b fragments. C3b is a potent opsonin, whereas C3a and C5a (derived from complement C5) each stimulate the oxidizing burst of macrophages as a part of inflammatory reactions. C5a also has chemotactic properties. Other acute phase reactants are produced by the
Immune Function in Diving

Liver. The immediate response to injury is a decrease in the circulating concentrations of these proteins, but if the pathologic process continues, hepatic production and thus plasma concentrations of acute phase reactants may rise.

Exposure of humans to HBO₂ (280 kPa) has no effect on complement levels in experienced divers (50). However, it has been suggested that decompression illness causes protein to be denatured at the bubble–plasma interface, and that the immune system then recognizes this as a foreign protein (51). Some studies have reported a modest activation of complement after dives (52,53). Guinea pigs show immediate activation, with a resulting small (25%) decrease in complement levels, followed by an increase 48 h after treatment (54). Moreover, the extent of such activation has been linked to the appearance of symptoms of decompression illness (52,53) both in rabbits (55,56) and in humans (57–59). Complement activation can take place not only in vivo, but also in vitro if protein denaturation is caused by the generation of either air or nitrogen bubbles in blood or serum (51,55,58,60).

One report found an increase in complement fraction C3 as trainees progressed through a 12-wk simulated diving course (61). Increased levels of C3a and C5a were correlated with the onset of type I decompression illness (joint pain only) in 3 of 11 divers performing a 28-day saturation dive, but values did not increase in 2 other divers who developed the high pressure nervous syndrome (57). A second study of 19 professional divers who performed repeated heliox dives found no correlation between C3a and C5a concentrations and susceptibility to decompression illness (62). Others have noted a decrease in both iC3 (a conformational change in C3 which is a prelude to activation of complement via the alternative pathway) and C3a for up to 24 h after standard decompression from a 58-min exposure to a simulated depth of 400 m (63). Since C3 levels did not change, it was argued that hemodilution was unlikely to be a factor; a binding of iC3 and C3a on activated cells was suggested, although the authors did not rule out the possibility that more severe or repeated bouts of diving might initiate a complement activation of the type reported by Stevens and associates (57).

A study of diving trainees noted a progressive increase in the plasma concentration of several other acute phase reactants (alpha-1-antitrypsin, acid glycoprotein, haptoglobin, and particularly complement fraction C3c in a week where bends developed) (61).

Some animal studies have suggested that acclimatization develops after many deep dives, with a parallel between a reduced activation of complement and a lesser susceptibility to decompression illness (64).

Clinical Evidence of Immunosuppression

Clinical evidence of overall immunosuppression may be sought in terms of an increased susceptibility to infections, a diminished response to antigens, a slower progression of autoimmune disorders and rejection of allografts, and an increased risk of genetic damage and neoplasic change.

Critique of data: Much of the available information is far from optimal. Few human studies have been able to adopt a randomized, double-blind controlled design. Often subjects have served as their own controls, with a potential for immune responses to be modified by seasonal factors or habituation to a given experimental situation, or both. Because of the high capital and operating costs of hyperbaric and deep-diving facilities, the number of subjects tested has often been small. Finally, those tested have usually been drawn from specialized populations—experienced divers, those undergoing diving training, or those with conditions for which HBO₂ is clinically useful.

Infections: Natural killer cells provide an important first line of defense against upper respiratory infections, and a normal action of phagocytic polymorphs is important to subsequent protection (7). However, susceptibility of a diver to viral and bacterial infections may reflect not only a disturbance of such immune mechanisms, but also exposure to environmental conditions which increase the risk of disease transmission, particularly high levels of humidity and life at close quarters with other diving personnel (65).

The use of He mixtures may also increase the metabolic activity of intrapulmonary cells, increasing the rate of viral replication (66). Indeed, He mixtures have been shown to cause a modest increase in the rate of growth of certain viruses in cell culture (67). Finally, divers may be exposed to water that has a high bacterial content, increasing the risk of ear and gastrointestinal infections (27,68,69).

Controlled studies of mice experimentally infected with influenza virus (66) and bacteria such as klebsiella pneumonia (14,70) have shown increased susceptibility during exposure to hyperbaric He–O₂ mixtures. Manifestations have included a greater consolidation of the lungs (66), greater infective titers (66), and an earlier median time to death (14). There is an associated depression of phagocytic indices (14), which seems linked to hypothermic stress and a resulting large increase in catecholamine secretion (14). However, some other mechanism also seems to be at work, since viral susceptibility remains high even if heat loss is normalized by increasing the chamber temperature to 35°C (66).

Findings in human subjects are not entirely consistent. General health appears good in naval personnel, who dive
relatively infrequently and observe cautious decompression schedules. A cross-sectional comparison of 11,517 US Navy divers with non-diving control found few disorders (except DCL) where hospitalization rates were higher for the divers (71). Moreover, in those over the age of 41 yr, there were no differences of morbidity or mortality between the two groups. A further study of 1977 US Navy diving officers found no health risks other than an excess of hospital admissions for cardiovascular disease and related stress disorders (72). In contrast, some small and uncontrolled studies of commercial divers have suggested an increased susceptibility to respiratory tract viruses and bacterial infections of the external ear (73–75). In addition to issues of experimental design, other variables include the frequency and duration of dives, and the type of invading microorganism; HBO₂ is an effective remedy against anaerobic bacteria.

Response to antigens: Cutaneous reactions to standard antigens provide a second index of cell-mediated immune responses that depend strongly on macrophage function. A decrease in skin reactions to PHA, staphylococcal toxin, polyvaccine, and tuberculin has been observed during the final weeks of diving training (30). At first inspection this suggests a diving-induced depression of the immune response but, in fact, the underlying mechanism might be a seasonal variation in immune responsiveness. Alternatively, immune function may have been suppressed by some other facet of the diving course [for example, repeated bouts of strenuous exercise and rigorous physical training (7)], rather than exposure to hyperbaric conditions per se.

Experiments with small mammals provide more convincing evidence that HBO₂ reduces immune responses. Contact sensitivity is decreased by 5-h daily exposures of mice to 250 kPa of O₂ for 4 days before or 5 days after sensitization to dinitrofluorobenzene (DNFB) (2). Hypersensitivity reactions to tuberculin are also delayed (4), and the generation of sheep erythrocyte antibodies in mice is decreased (6).

The normal DNFB response can be restored by administration of peritoneal exudate, but not by lymph node cells (4), supporting the view that a decrease in macrophage function is responsible for these changes.

Autoimmune disorders: In accord with the animal studies of antigen responses, exposure to HBO₂ suppresses experimental allergic encephalomyelitis (EAE) (4,76,77) and adjuvant arthritis (78) in rodents. Exposure to hyperbaric conditions (6 h · day⁻¹ at 200 kPa, beginning 1–6 days after the injection of myelin) completely suppressed EAE in all of 107 test guinea pigs, as compared with an incidence of 100/112 in control animals (4). Later and less prolonged O₂ administration (2 h · day⁻¹ at 250 kPa, beginning 5–19 days after inoculation) still slowed the onset of EAE by 4–5 days relative to control animals (76). The development of autoimmune disorders in the mouse (proteinuria, facial erythema, and lymphadenopathy) showed a similar (but statistically non-significant) trend following long-term hyperbaric treatment [253 kPa 1 h · day⁻¹ for 2 mo. (6)].

Hyperbaric oxygen has also been proposed for the treatment of multiple sclerosis (a human demyelinating disease), although its use is controversial (79,80). In at least one study of such patients, immune function was actually enhanced by a period of hyperbaric treatment (81). There are various possible explanations of the discrepancy in response between EAE and multiple sclerosis. In EAE, the inflammatory process precedes demyelination, whereas the converse is true in multiple sclerosis. The HBO₂ may also be acting through changes in metabolism of myelin or glucose, or through a reduction of central edema rather than by an effect on the immune system. Finally, a moderate dose of O₂ may stimulate immune responses, whereas a more challenging exposure or a series of exposures has an immunosuppressant effect.

Allografts: Further supporting the inference that exposure to HBO₂ can have an immunosuppressant effect on small mammals, numerous studies have shown that such treatment slows but does not prevent the rejection of allografts such as skin and thyroid tissue (82–88).

For example, Erdmann et al. found that low and moderate exposures to 250 kPa O₂ (2 × 90 min, once per week, and 2 × 90 min 3 times per week) delayed the onset of rejection of a potently immunogenic skin graft from 7.0 to 7.8 and 9.0 days, respectively. Likewise, Jacobs et al. extended the survival of an ear allograft by administering 200 kPa of O₂ 6 h · day⁻¹.

Genetic change and susceptibility to neoplasia: The impact of HBO₂ on the risk of cancer among divers has recently been reviewed (89). A few investigators have suggested that hyperbaric conditions can cause or facilitate the growth and metastasis of neoplasms (90), but both animal and human studies provide many more negative than positive reports; moreover, trials where adverse effects were reported have in general been negated by subsequent larger and more sophisticated studies (89).

If there are some circumstances where HBO₂ can have an adverse effect, this may be because the delivery of O₂ to rapidly growing and poorly perfused tissue is enhanced, rather than because the high partial pressures of O₂ cause an increase of free radicals and/or an immunosuppression that facilitate tumor growth and metastasis.

In terms of genetic change, murine studies have suggested that hyperbaric conditions can lead to sub-fertility (91–93), and there have been occasional reports of persistent azoospermia in divers (94). An uncontrolled survey (95)
found that in a small proportion of professional divers (6 of 77 compressed air and 76 mixed gas divers) a few T cells had a heavily damaged chromosomal structure. The authors linked this observation to the discovery of triploid zygotes in the child of a diver, but since only a single child was involved, this seems likely to be a chance finding rather than a consequence of diving. Further, any health risk from the chromosomal aberrations is probably small, since the T cell abnormalities were sufficiently gross that the cells concerned would probably have died at mitosis. In support of this view, a follow-up study suggested that the damaged cells disappeared quickly from the circulation (93).

MECHANISMS OF IMMUNE CHANGE

Exposure to microorganisms: There is often an increased exposure to respiratory, ear, and cutaneous infection in the deep-water environment, and some reactions such as a neutrocytosis may be secondary to such infection.

Hormonal changes: Enclosure in a hyperbaric chamber is a frightening experience for many patients, and deep dives can also be stressful (96). One would thus anticipate that exposures might cause a typical stress hormone response (the release of catecholamines, and subsequently of cortisol), with consequent implications for the mobilization, recruitment, vascular egress, and functional activity of the various leukocyte subsets (7). In general, habituation would diminish such reactions with repetition of the exposure. Such effects may be exacerbated by more direct hormonal responses to high pressures, He gas mixtures, and associated hypothermia (9). For example, mice maintained at 8 atm pressure (97% He, 3% O₂) show a sustained, 3-fold increase in circulating levels of catecholamines (14). Bubble formation in the cerebral circulation, and resultant obstruction of the blood flow to the hypothalamic centers may also augment catecholamine production (97). A decrease in serum cortisol has been seen following decompression (18), but changes with HBO₂ are minimal (86). The production of prostaglandin E by macrophages (3) and brain tissue (98) is also decreased by exposure to HBO₂.

The interaction of these various hormonal changes is complex. The increased concentration of catecholamines spurs the circulating mobilization of leukocytes (7). NK cell activity is enhanced, but the phagocytic activity of the neutrophils is depressed (14). Cortisol stimulates a circulatory influx of neutrophils from the bone marrow, but also depresses the activity of circulating NK cells; the post-decompression decrease in serum cortisol would presumably have the opposite effect. Excessive concentrations of prostaglandin E2 also suppress NK cell activity, in part by inhibiting the production of IL-1 and IL-6.

Acclimatization occurs with repetition of deep diving (99). It seems logical to attribute much of this response to habituation and a reduction in psychologic stress.

Reactive species: A second factor influencing immune responses in the hyperbaric environment could be an increased concentration of reactive species. Beta-carotene certainly protects the central nervous system against the adverse effects of high O₂ pressures (100). Reactive species are toxic for many types of cell, and can cause a selective depletion of lymphocyte subsets (101). The lungs are particularly vulnerable to hyperoxia, and the mitochondrial contribution to free radical production is augmented 15- to 20-fold under hyperoxic conditions (42). Reactive species have a very short half-life, but any resulting tissue damage is likely to be cumulative, depending on the number of treatments that are given and their duration.

However, animal experiments suggest that prolonged exposure to HBO₂ also leads to an increased activity of the free radical scavenger superoxide dismutase, sometimes with a net decrease in free radical exposure (36,37,102). Moreover, animals who have developed pulmonary edema secondary to smoke inhalation respond favorably to HBO₂, despite initial pulmonary damage and free radical formation (103). This suggests that the main loading of reactive species may come from the phagocytes, rather than from some other effect of HBO₂ (104).

The physical activity required by diving may exacerbate the effect of a given increase in partial pressure of O₂, but nevertheless the build up of reactive species is likely to be smaller than during HBO₂. Reactive species remains one possible explanation of any chromosomal abnormalities that may be induced by diving, but it remains important to exclude other potential causes of DNA damage, such as the use of x-rays and radioactive cobalt sources by divers when they are examining and repairing underwater structures (94).

Leukocyte redistribution: The rapid reversal of leukocyte changes after exposure suggests that changes in cell numbers reflect a redistribution of cells rather than selective cell death. In some instances, cells are migrating to the lungs in response to injury of the pulmonary tissues. The pulmonary migration of neutrophils can be reduced by administering the xanthine oxidase inhibitor Allopurinol (105), which is thought to block the local inflammatory response.

Local tissue reactions: Some of the observed responses seem secondary to local tissue reactions to injury and bubble formation. Some authors have found significant changes in complement fractions during diving and subsequent decompression. One study found the largest increase was in complement fraction C3, which is related to inflammatory processes; fraction C4 (which is more closely
linked to immune reactions) showed much smaller changes (61). This observation supported the view that the accumulation of complement reflected an acute phase response to local inflammation and/or vascular damage during decompression. Another study found an increased production of complement fraction C5a, which is a strong stimulant of neutrophil aggregation (57). The complement fractions C3a and C5a are also potent anaphylotoxins, and could contribute to the clinical picture of decompression illness by provoking histamine release from mast cells. C5a is also chemotactic for neutrophils, stimulating the formation of O₂ radicals and increasing their adherence to endothelial cells (106,107).

The development of decompression illness is also associated with an increased aggregation of the blood constituents, both platelets and leukocytes (5,108). End (109) considered such aggregation to be the primary event in decompression illness. It could arise from an up-regulation of adhesion molecules but it could also be secondary to protein denaturation, complement activation, non-specific inflammation (110), and other events occurring at either the bubble interface or the damaged endothelial lining of the blood vessels. Aggregation of platelets and their adherence to bubbles cause the release of modulators of both endothelial and leukocyte function (111) and secondary alterations of regional blood flow (112,113). In addition to such indirect activation, there is growing evidence that bubble formation can activate granulocytes directly (5,108,114,115).

A lessening of response is seen with repeated decompression of animals such as the rabbit. This is associated with a reduced activation of complement (56,64), although it is less clear which is cause and which effect.

CLINICAL IMPLICATIONS

The few hours of immunosuppression associated with a single dive are unlikely to have great clinical significance, but the continued suppression of T cell function over a 30-day bout of saturation diving could theoretically increase susceptibility to both infections and neoplastic cells. Nevertheless, available epidemiologic data do not suggest any significant clinical problems. This may be in part because those who are engaged in diving on a regular basis develop compensatory changes.

The situation seems to be more critical during the clinical use of HBO₂. In many instances, the patient is sick, with an established anaerobic infection or tumor, the partial pressure of O₂ in the treatment chamber is high, the “stress” of therapy is probably greater than in a routine diving operation, and exposures do not continue over sufficient time to allow acclimatization. A careful watch must thus be kept for any immunosuppression that could exacerbate infections and/or speed tumor growth.

The reported correlations between decompression illness and immune changes have considerable scientific interest, but to date the associations have been relatively weak, and much more investigation is needed before immune responses can be used to predict an individual’s susceptibility to decompression illness.

RESEARCH NEEDS

There remains a need to clarify mechanisms responsible for any changes of immune response observed during and after HBO₂ treatment and deep diving. This should focus on the respective roles of high ambient pressures and associated changes in the partial pressure of O₂ during exposure, and (in the case of the divers) on bubble formation during decompression. Given that physical activity itself modifies immune function (7), it will be necessary to consider the effects of bed rest in those receiving hyperbaric treatment, and in diving to distinguish trials that demand little physical work from those that demand vigorous exercise and/or physical training. Further comparison of hyperbaric treatment with responses to short- and long-term dives and the use of new techniques to detect bubble formation may help to elucidate mechanisms. Finally, if any of the observed changes prove to have clinical importance, dose–response relationships will need to be studied, with the goal of developing immunologically “safe” hyperbaric treatment and diving profiles.

CONCLUSIONS

Exposure to HBO₂, deep diving, and decompression can all induce immediate alterations in immune function. Changes involving T cells, macrophages, neutrophils, complement, and acute phase reactants suggest some net reduction in immune response, but effects are generally short-lived. In professional divers, adaptive reactions may give at least partial compensation, and changes seem to have little clinical significance. However, when HBO₂ is used for clinical purposes, an anaerobic infection or tumor may already weaken the patient, and a careful monitoring of immune responses may be important to the efficacy of treatment.

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